From the INTERNATIONAL BUREAU

PCT

NOTIFICATION OF ELECTION

(PCT Rule 61.2)

To:

Commissioner
US Department of Commerce
United States Patent and Trademark
Office, PCT
2011 South Clark Place Room
CP2/5C24
Arlington, VA 22202
ETATS-UNIS D'AMERIQUE

in its capacity as elected Office

Date of mailing (day/month/year) 08 May 2001 (08.05.01)	
International application No. PCT/EP00/08088	**Applicant's or agent's file reference** 20373P WO
International filing date (day/month/year) 18 August 2000 (18.08.00)	**Priority date** (day/month/year) 20 August 1999 (20.08.99)
Applicant TISCHER, Wilhelm et al	

1. The designated Office is hereby notified of its election made:

   [X] in the demand filed with the International Preliminary Examining Authority on:

   26 February 2001 (26.02.01)

   [ ] in a notice effecting later election filed with the International Bureau on:

2. The election [X] was

   [ ] was not

   made before the expiration of 19 months from the priority date or, where Rule 32 applies, within the time limit under Rule 32.2(b).

The International Bureau of WIPO
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Authorized officer

Christine Carrié

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PATENT COOPERATION TREATY

From the
INTERNATIONAL PRELIMINARY EXAMINING AUTHORITY

To:

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Weickmann & Weickmann
PCT E 23. JAN. 2002
Frist:
Patentanwälte

NOTIFICATION OF TRANSMITTAL OF THE INTERNATIONAL PRELIMINARY EXAMINATION REPORT
(PCT Rule 71.1)

Date of mailing *(day/month/year)*	21.01.2002
Applicant's or agent's file reference: 20373P WO	**IMPORTANT NOTIFICATION**

International application No.	International filing date *(day/month/year)*	Priority date *(day/month/year)*
PCT/EP00/08088	18/08/2000	20/08/1999

Applicant: ROCHE DIAGNOSTICS GMBH et al.

1. The applicant is hereby notified that this International Preliminary Examining Authority transmits herewith the international preliminary examination report and its annexes, if any, established on the international application.

2. A copy of the report and its annexes, if any, is being transmitted to the International Bureau for communication to all the elected Offices.

3. Where required by any of the elected Offices, the International Bureau will prepare an English translation of the report (but not of any annexes) and will transmit such translation to those Offices.

4. **REMINDER**

The applicant must enter the national phase before each elected Office by performing certain acts (filing translations and paying national fees) within 30 months from the priority date (or later in some Offices) (Article 39(1)) (see also the reminder sent by the International Bureau with Form PCT/IB/301).

Where a translation of the international application must be furnished to an elected Office, that translation must contain a translation of any annexes to the international preliminary examination report. It is the applicant's responsibility to prepare and furnish such translation directly to each elected Office concerned.

For further details on the applicable time limits and requirements of the elected Offices, see Volume II of the PCT Applicant's Guide.

Name and mailing address of the IPEA/	Authorized officer
European Patent Office D-80298 Munich Tel. +49 89 2399 - 0 Tx: 523656 epmu d Fax: +49 89 2399 - 4465	Cleere, C Tel.+49 89 2399-7713

Form PCT/IPEA/416 (July 1992)

PATENT COOPERATION TREATY

PCT

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Article 36 and Rule 70)

Applicant's or agent's file reference	FOR FURTHER ACTION	See Notification of Transmittal of International Preliminary Examination Report (Form PCT/IPEA/416)
20373P WO		
International application No.	International filing date *(day/month/year)*	Priority date *(day/month/year)*
PCT/EP00/08088	18/08/2000	20/08/1999
International Patent Classification (IPC) or national classification and IPC C12N15/54		
Applicant ROCHE DIAGNOSTICS GMBH et al.		

1. This international preliminary examination report has been prepared by this International Preliminary Examining Authority and is transmitted to the applicant according to Article 36.

2. This REPORT consists of a total of 7 sheets, including this cover sheet.

   ☐ This report is also accompanied by ANNEXES, i.e. sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications made before this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions under the PCT).

   These annexes consist of a total of 7 sheets.

3. This report contains indications relating to the following items:

   - I ☒ Basis of the report
   - II ☐ Priority
   - III ☐ Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
   - IV ☒ Lack of unity of invention
   - V ☒ Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations suporting such statement
   - VI ☐ Certain documents cited
   - VII ☐ Certain defects in the international application
   - VIII ☐ Certain observations on the international application

Date of submission of the demand	Date of completion of this report
26/02/2001	21.01.2002
Name and mailing address of the international preliminary examining authority: European Patent Office D-80298 Munich Tel. +49 89 2399 - 0 Tx: 523656 epmu d Fax: +49 89 2399 - 4465	Authorized officer Heckl, K Telephone No. +49 89 2399 8430

Form PCT/IPEA/409 (cover sheet) (January 1994)

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No. PCT/EP00/08088

I. Basis of the report

1. With regard to the **elements** of the international application *(Replacement sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to this report since they do not contain amendments (Rules 70.16 and 70.17))*:
Description, pages:

1-25	as originally filed				

Claims, No.:

1-45	as received on	25/09/2001	with letter of	25/09/2001	

Drawings, sheets:

1/4-4/4	as originally filed

Sequence listing part of the description, pages:

1-24, as originally filed

2. With regard to the **language**, all the elements marked above were available or furnished to this Authority in the language in which the international application was filed, unless otherwise indicated under this item.

   These elements were available or furnished to this Authority in the following language: , which is:

   - ☐ the language of a translation furnished for the purposes of the international search (under Rule 23.1(b)).
   - ☐ the language of publication of the international application (under Rule 48.3(b)).
   - ☐ the language of a translation furnished for the purposes of international preliminary examination (under Rule 55.2 and/or 55.3).

3. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the international preliminary examination was carried out on the basis of the sequence listing:

   - ☒ contained in the international application in written form.
   - ☒ filed together with the international application in computer readable form.
   - ☐ furnished subsequently to this Authority in written form.
   - ☐ furnished subsequently to this Authority in computer readable form.
   - ☐ The statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.
   - ☐ The statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished.

4. The amendments have resulted in the cancellation of:

- ☐ the description, pages:
- ☐ the claims, Nos.:
- ☐ the drawings, sheets:

5. ☐ This report has been established as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed (Rule 70.2(c)):

   (Any replacement sheet containing such amendments must be referred to under item 1 and annexed to this report.)

6. Additional observations, if necessary:

IV. Lack of unity of invention

1. In response to the invitation to restrict or pay additional fees the applicant has:

   - ☐ restricted the claims.
   - ☐ paid additional fees.
   - ☐ paid additional fees under protest.
   - ☒ neither restricted nor paid additional fees.

2. ☐ This Authority found that the requirement of unity of invention is not complied and chose, according to Rule 68.1, not to invite the applicant to restrict or pay additional fees.

3. This Authority considers that the requirement of unity of invention in accordance with Rules 13.1, 13.2 and 13.3 is

   - ☐ complied with.
   - ☐ not complied with for the following reasons:

4. Consequently, the following parts of the international application were the subject of international preliminary examination in establishing this report:

   - ☐ all parts.
   - ☒ the parts relating to claims Nos. 1-10 and 45, all partially.

V. Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

   Novelty (N) Yes: Claims 1-10, all partially

	No:	Claims	45, partially
Inventive step (IS)	Yes:	Claims	
	No:	Claims	1-10 and 45, all partially
Industrial applicability (IA)	Yes:	Claims	1-10, 45, all partially
	No:	Claims	

2. Citations and explanations
see separate sheet

Re Item IV

Lack of unity of invention

1. The international search report has been drawn up in respect of claims 1-10 (1-11 as originally filed) and 45 part. (46 part. as originally filed) of the international application but the International Preliminary Examining Authority is of the opinion that also this part of the application does not comply with the requirements of unity of invention as set forth in the PCT regulations (Article 34(3), Rules 13 and 68 PCT).

2. The separate inventions/groups of invention are:

   A method for the in vitro enzymatic synthesis of deoxyribonucleosides comprising reacting deoxyribose 1-phosphate and a nucleobase and inorganic phosphate is formed and removed (claim 1) wherein the reaction is catalysed by a thymidine phosphorylase or a purine nucleoside phosphorylase (claim 2), and wherein the nucleobase is selected from thymine, uracil, adenine, guanine, hypoxanthine and analogs thereof selected from those as indicated in claim 3.

   Regarding the 11 nucleobases indicated in claim 3 the method of claims 1-3 embraces 11 different inventions.

3. They are not so linked as to form a single general inventive concept for the following reasons:

   The common concept linking together the independent claims is a method for the in vitro enzymatic synthesis of deoxyribonucleosides comprising reacting deoxyribose 1-phosphate and a nucleobase and inorganic phosphate being formed and removed (claim 1).

   However, a method of deoxynucleoside synthesis is not novel as becomes evident from D2, Fig.3a in connection with the description related thereto. Also the feature of removing an end product from an enzyme catalysed reaction cannot be considered as an inventive contribution (see also section V).

The common concept underlying claim 3 was to exemplify the teaching of claims 1 and 2 with respect to one or more particular nucleobases which concept is also known from the above document, page 262, last paragraph, page 263, first paragraph and page 265, "Conclusion".

Taken together there is no common and inventive concept linking together each of the embodiments referred to in claim 3. Accordingly, each thereof represents a single invention.

4. Since the Applicant neither restricted the claims nor payed additional fees the subject-matter first referred to in claim 3 (the nucleobase is thymine) has been selected as the subject of the IPER.

In detail, this subject-matter is the teaching of independent claims 1 and 45 having regard to thymine as the base according to claim 3 and to thymidine phosphorylase as the enzyme catalysing the reaction.

Re Item V

Reasoned statement under Rule 66.2(a)(ii) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

The following documents are referred to:

D1: EP-A-0 411 158 (YAMASA SHOYU KK) 6 February 1991 (1991-02-06)

D2: C.F. BARBAS III AND C.-H. WONG: 'Overexpression and substrate specificity studies of Phosphodeoxyribomutase and thymidine phosphorylase' BIOORGANIC CHEMISTRY, vol. 19, no. 3, September 1991 (1991-09), pages 261-269, XP000867811 ACADEMIC PRESS, NY,US

D3: M. FISCHER AND S.A. SHORT: 'The cloning of the Escherichia coli L-12 deoxyribonucleoside operon' GENE, vol. 17, no. 3, March 1982 (1982-03), pages 291-298, XP000867826 ELSEVIER SCIENCE PUBLISHERS,B.V.,AMSTERDAM,NL;

Novelty (Art.33(2) PCT):

D1 (see page 9, line 36 to page 10, line 20; page 11, line 29 to page 12, line 9; claim 3) and D2 (se Fig.3a and the description related thereto) disclose the process of present claims 1-3 having regard to thymine as the nucleobase with the exception of the removal of phosphate. Therefore, claims 1-3 and claims 4-10 are formally considered novel.

D3 (see Introduction, Abstract, Results (b) and Table 1) discloses the **deo** operon and E.coli transformants containing the same or fragments thereof such as the **deoA** gene encoding TP (plasmid/clone pMFS50). Transformant pMFS50 also contains a gene encoding TP. Since there are no features evident which allow do discriminate between transformant I-2186 of present claim 45 and prior art clone pMFS50, novelty of present claim 45 cannot be acknowledged.

Inventive step (Art.33(3) PCT):

The feature of removing an end product from an enzyme catalysed reaction cannot be considered as an inventive contribution. In fact, it is common general knowledge that removal of an end product moves the reaction equilibrium towards its end products which effect is desirable in any method of synthesis.

Furthermore, and in order to exemplify the teaching of removing phosphate the skilled person could select among various possibilities including those of claims 4-10, either. Therefore, claims 1-10 cannot be considered inventive.

Taken the case that novelty of I-2186 could be acknowledged it remains questionable whether said transformant could be considered inventive. In fact, D3 describes the location of the **deoA** gene within the **deo** operon so that the skilled person is clearly advised and enabled to clone the same (see D3, page 295, right hand column, lines 15-17).

Claims

1. A method for the in vitro enzymatic synthesis of deoxyribonucleosides comprising reacting deoxyribose 1-phosphate (dR1P) and a nucleobase, wherein a deoxyribonucleoside and inorganic phosphate are formed.

2. The method of claim 1, wherein the inorganic phosphate is removed.

3. The method of claim 1 or 2, wherein the reaction is catalyzed by a thymidine phosphorylase (TP, EC 2.4.2.4) or a purine nucleoside phosphorylase (PNP, EC 2.4.2.1).

4. The method of any one of the previous claims, wherein the nucleobase is selected from the group consisting of thymine, uracil, adenine, guanine and hypoxanthine and analogs thereof, e.g. 2-thio-uracil, 6-aza-uracil, 5-carboxy-2-thio-uracil, 6-aza-thymine, 6-aza-2-thio-thymine and 2,6-diamino-purine.

5. The method of any one of the previous claims, wherein the removal of the inorganic phosphate is effected by (i) conversion to inorganic pyrophosphate, (ii) precipitation, (iii) complexation and/or (iv) substrate phosphorylation.

6. The method of claim 5, wherein the inorganic phosphate is converted to pyrophosphate by a phosphate transfer from fructose-diphosphate (FDP) under formation of fructose-6-phosphate (F6P).

7. The method of claim 6, wherein the phosphate transfer is catalyzed by a PPi-dependent phosphofructokinase (PFK-PPi, EC 2.7.1.90).

8. The method of claim 6 or 7, wherein the inorganic pyrophosphate is removed by precipitation.

9. The method of claim 5, wherein the inorganic phosphate is transferred to a disaccharide, particularly sucrose or maltose under formation of a monosaccharide and a phosphorylated monosaccharide.

10. The method of claim 9, wherein the phosphate transfer is catalyzed by a sucrose phosphorylase (EC 2.4.1.7) or a maltose phosphorylase (EC 2.4.1.8).

11. The method of claim 10, wherein the phosphorylated monosaccharide is further reacted.

12. The method of any one of the previous claims, wherein the deoxyribose-1-phosphate is generated from deoxyribose 5-phosphate (dR5P).

13. The method of claim 12, wherein the reaction is catalyzed by a deoxyribomutase (EC 2.7.5.1) or a phosphopentose mutase (PPM, EC 5.4.2.7).

14. The method of claim 12 or 13, wherein the deoxyribose-5-phosphate is generated by a condensation of glyceraldehyde 3-phosphate (GAP) with acetaldehyde.

15. The method of claim 14, wherein the reaction is catalyzed by a phosphopentose aldolase (PPA, EC 4.1.2.4).

16. The method of claim 14 or 15, wherein the glyceraldehyde 3-phosphate is generated from fructose 1,6-diphosphate, dihydroxyacetone (DHA) and/or glycerolphosphate.

17. The method of claim 16, wherein the glyceraldehyde 3-phosphate is generated from fructose 1,6-diphosphate in a reaction catalyzed by an FDP-aldolase (EC 4.1.2.13) selected from FDP-aldolases I and FDP-aldolases II.

18. The method of claim 16, wherein the glyceraldehyde 3-phosphate is generated from dihydroxyacetone and ATP under formation of dihydroxyacetone phosphate (DHAP) and ADP and subsequent isomerization of DHAP to GAP in a reaction catalyzed by a glycerokinase (GK, EC 2.7.1.30) and a triose phosphate isomerase (TIM, EC 5.3.1.1).

19. The method of claim 16, wherein the glyceraldehyde 3-phosphate is generated from glycerol phosphate (GP) and O_2 under formation of dihydroxyacetone phosphate (DHAP) and H_2O_2 and subsequent isomerization of DHAP to GAP in a reaction catalyzed by a glycerophosphate oxidase (GPO, EC 1.1.3.21) and a triose phosphate isomerase (TIM, EC 5.3.1.1).

20. The method of claim 12 or 13, wherein the deoxyribose 5-phosphate is generated by a phosphorylation of deoxyribose.

21. The method of claim 20, wherein the reaction is catalyzed by a deoxyribokinase (dRK, EC 2.7.1.15).

22. The method of claim 21, wherein a dRK obtainable from Salmonella typhi is used which is encoded by (a) the nucleotide sequence shown in SEQ ID NO.11 or its complementary sequence, (b) a nucleotide sequence corresponding to the sequence of (a) in the scope of the degeneracy of the genetic code or (c) a nucleotide sequence hybridizing under stringent conditions to the sequence of (a) and/or (b).

23. The method of any one of the previous claims, wherein a deoxyribonucleoside containing a first nucleobase is further reacted with a second nucleobase under formation of a deoxyribonucleoside containing the second nucleobase.

24. The method of claim 23, wherein said second nucleobase is selected from cytidine and analogs thereof, e.g. 5-aza-cytidine, 2,6-dichloro-purine, 6-chloro-guanine, 6-chloro-purine, 6-aza-thymine and 5-fluoro-uracil.

25. The method of claim 24, wherein the reaction is catalyzed by a nucleoside 2-deoxyribosyl transferase (NdT, EC 2.4.2.6).

26. The method of claim 25, wherein an NdT obtainable from Lactobacillus leichmannii is used which is encoded by (a) the nucleotide sequence shown in SEQ ID NO.13 or its complementary sequence, (b) a nucleotide sequence corresponding to the sequence of (a) in the scope of the degeneracy of the genetic code or (c) a nucleotide sequence hybridizing under stringent conditions to the sequence of (a) and/or (b).

27. A method for the in vitro enzymatic synthesis of deoxyribonucleosides comprising the steps of:

(i) condensing glyceraldehyde 3-phosphate (GAP) with acetaldehyde to deoxyribose 5-phosphate (dR5P),

(ii) isomerizing deoxyribose 5-phosphate to deoxyribose 1-phosphate (dR1P) and

(iii) reacting deoxyribose 1-phosphate and nucleobase, wherein a deoxyribonucleoside and inorganic phosphate are formed.

28. The method of claim 27, wherein the reaction is carried out without isolating intermediate products.

29. The method of claim 27 or 28, wherein the glyceraldehyde 3-phosphate (GAP) is generated from fructose 1,6-diphosphate (FDP), dihydroxy-acetone (DHA) and/or glycerolphosphate (GP).

30. The method of claims 27 to 29, wherein before step (ii) excess acetaldehyde is removed.

31. The method of claims 27 to 30, wherein before step (ii) excess starting materials and/or by-products, particularly fructose 1,6-diphosphate and/or deoxyxylulose 1-phosphate (dX1P) are removed.

32. The method of claims 27 to 30, wherein the reaction is carried out in a manner that before step (ii) no substantial amounts of starting materials and/or by-products, particularly fructose 1,6-diphosphate and/or deoxyxylulose 1-phosphate are present.

33. A method for the in vitro enzymatic synthesis of deoxyribonucleosides comprising the steps of:

(i) phosphorylating deoxyribose to deoxyribose 5-phosphate,

(ii) isomerizing deoxyribose 5-phosphate to deoxyribose 1-phosphate and

(iii) reacting deoxyribose 1-phosphate and nucleobase, wherein a deoxyribonucleoside an inorganic phosphate are formed.

34. The method of claim 33, wherein the reaction is carried out without isolating intermediate products.

35. The method of claims 27 to 34, wherein the inorganic phosphate is removed.

36. The method of any one of the previous claims comprising further reacting said deoxyribonucleoside.

37. The method of claim 36, wherein said further reacting comprises the synthesis of deoxyribonucleoside mono-, di- or triphosphates, of H-phosphonates or of phosphoramidites.

38. The use of an isolated nucleic acid molecule encoding a nucleoside 2-deoxyribosyl transferase (NdT, EC 2.4.2.6) for the preparation of an enzyme for an in vitro method for the enzymatic synthesis of deoxyribonucleosides, wherein a deoxyribonucleoside containing a first nucleobase is further reacted with a second nucleobase under formation of a deoxyribonucleoside containing the second nucleobase, wherein said nucleic acid molecule comprises (a) the nucleotide sequence shown in SEQ ID NO.13 or its complementary sequence, (b) a nucleotide sequence corresponding to the sequence of (a) in the scope of the degeneracy of the genetic code or (c) a nucleotide sequence hybridizing under stringent conditions to the sequence of (a) and/or (b).

39. The use of claim 38, wherein the second nucleobase is selected from cytidine and analogs thereof, e.g. 6-methyl purine, 2-amino-6-methylmercaptopurine, 6-dimethylaminopurine, 5-azacytidine, 2,6-dichloropurine, 6-chloroguanine, 6-chloropurine, 6-azathymine, 5-fluorouracil, ethyl-4-amino-5-imidazole carboxylate, imidazole-4-carboxamide and 1,2,4-triazole-3-carboxamide.

40. The use of claim 38 or 39, wherein the first nucleobase is selected from adenine, guanine, thymine, uracil and hypoxanthine.

41. The use of any one of claims 38-40, wherein the nucleic acid molecule is contained on a recombinant vector in operative linkage with an expression control sequence.

42. The use of any one of claims 38-41, wherein the nucleic acid is contained in a recombinant cell.

43. Use of an isolated polypeptide having NdT activity for the preparation of nucleosides according to claim 24.

44. Use of an isolated nucleic acid molecule encoding a deoxyribokinase (dRK, EC 2.7.1.5) for the preparation of an enzyme for an in vitro method for the enzymatic synthesis of deoxyribonucleosides comprising the step of phosphorylating deoxyribose to deoxyribose 5-phosphate, wherein said nucleic acid molecule comprises (a) the nucleotide sequence shown in SEQ ID NO.11 or its complementary sequence, (b) a nucleotide sequence corresponding to the sequence of (a) in the scope of the degeneracy of the genetic code or (c) a nucleotide sequence hybridizing under stringent conditions to the sequence of (a) and/or (b).

45. Use of an isolated polypeptide having dRK activity for an in vitro method for the enzymatic synthesis of deoxyribonucleosides comprising the step of phosphorylating deoxyribose to deoxyribose 5-phosphate.

46. Recombinant bacteria strains deposited at CNCM under accession numbers I-2186, I-2187, I-2188, I-2189, I-2190 and I-2191.